AMH

Anti-Mullerian Hormone (ELISA)

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REF: DS177700 Σ 96

Intended use
The DiaSino AMH assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative determination of Anti-Mullerian Hormone (AMH) in human serum. For professional use only.

Summary
Anti-Müllerian hormone (AMH) is a glycoprotein hormone structurally related to inhibin and activin from the transforming growth factor beta superfamily, whose key roles are in growth differentiation and folliculogenesis.[1]
In fetal males, AMH is produced by the Sertoli cells and induces regression of the Müllerian duct and therefore promotes development of the male reproductive tract.[2] Infant males have very high levels of AMH (>30 ng/ml) that slowly decreases until post-pubescence where it remains at a low level (<10 ng/ml).[3,4]
In females, AMH begins to be produced near the time of birth with levels increasing until puberty. After puberty, blood AMH levels decrease until menopause where it becomes nearly undetectable (<0.1 ng/ml). AMH concentration in female blood has repeatedly been linked to ovarian reserve, thereby giving an indication to patients' remaining reproductive lifespans. Additionaly, high levels of AMH (>4.7 ng/ml, 80% CI) in females are an indication of polycystic ovarian syndrome (PCOS).[5]
When AMH levels drop below 1.0 ng/ml in females, they are considered to have low ovarian reserves. Patients in these ranges are advised to not delay family planning or to undergo infertility treatments such as in vitro fertilization.[5,6]
The DiaSino AMH ELISA kit can be used to monitor the progress of patients' infertility treatments and approximate the onset of menopause.

Test principle
Sandwich principle. Total duration of assay: **80 minutes.**
- Sample, Anti-AMH coated microwells and enzyme labeled Anti-AMH are combined.
- During the incubation, AMH presents in the sample is allowed to react simultaneously with the two antibodies, resulting in the AMH molecules being sandwiched between the solid phase and enzyme-linked antibodies.
- After washing, a complex is generated between the solid phase, the AMH within the sample and enzyme-linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The absorbance is proportional to the amount of AMH in the sample.

Reagents
Materials provided
- **AMH Coated Microplate - symbol** AMH PLATE 8 x 12 strips, 96 wells, pre-coated with mouse monoclonal Anti-AMH.
- **AMH Calibrators - symbol** AMH CAL A-F 6 vials, 1 mL each, ready to use; Concentrations: 0(**A**), 0.5(**B**), 2(**C**), 5(**D**), 10(**E**) and 25(**F**) ng/mL.
- **AMH Enzyme Conjugate - symbol** AMH CONJ 1 vial, 6.0 mL of HRP (horseradish peroxidase) labeled mouse monoclonal Anti-AMH in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300 preservative.
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU -** 1 copy.
- **Plate Lid**: 1 piece.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Incident report
Any suspected serious incidents related to this assay shall be immediately reported to DiaSino, DiaSino's Authorized Representative in the EU, and the national competent authorities of the Member States where the users and/or patients are located.

Storage and stability
- Store at 2-8℃.
- Seal and return unused reagents to 2-8℃, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum is recommended for this assay.
- Cap and store the samples at 18-25 ℃ for no more than 8 hours. Stable for 7 days at 2-8℃, and 1 month at -20℃. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Calibration
The DiaSino AMH ELISA has been standardized against the National Institute for Biological Standards and Control (NIBSC) code 16/190.
Recalibration is recommended when a new reagent lot is used, or the quality controls are out of specified range.

Quality control
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate.
Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure
Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 ℃) before measurement. Mix all reagents through gently inverting prior to use.
- Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
- Add **50 µL** of **calibrators** or **samples** to each well.
- Add **50 µL** of **enzyme conjugate** to each well.
- Shake the **microplate** gently for **30 seconds** to mix.
- Cover the plate with a plate lid and incubate at **37 ℃** for **60 minutes**.
  Note: Incubation temperature between 37-40℃ is acceptable, but can not be lower than 37℃, otherwise the testing results will be lower!
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add **350 µL** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **100 µL** of **substrate** to each well.
- Cover and **Incubate** at **ambient temperature (18-25℃)** in the dark for reaction for **20 minutes**. Do not shake the plate after substate addition.
- Add **50 µL** of **stop solution** to each well.
- Shake for **15-20 seconds** to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
- Read the absorbance of each well at **450 nm** (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within **30 minutes** of adding the stop solution.

Calculation
- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in ng/mL for each calibrator.

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- Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Sample	Value (ng/mL)	Absorbance
Calibrator A	0	0.014
Calibrator B	0.5	0.133
Calibrator C	2	0.288
Calibrator D	5	0.868
Calibrator E	10	1.477
Calibrator F	25	2.932
Control 1	2.78	0.438
Control 2	13.30	1.797
Sample	3.70	0.617

Limitations - interference

- The assay is unaffected by icterus (bilirubin < 600 µmol/L or < 35 mg/dL), hemolysis (Hb < 0.559 mmol/L or < 0.9 g/dL), lipemia (Intralipid < 1200 mg/dL), and biotin < 94 nmol/L or < 23 ng/mL.
- Criterion: Recovery within ± 10 % of initial value.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
- Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
- The presence of autoantibodies may induce high molecular weight complexes (macro-AMH) which may cause unexpected high values of AMH.
- Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
- Serum AMH values may be elevated by pharmacological intervention. Domperiodone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase AMH levels.

Measuring range

0.02-25.0 ng/mL (defined by the lower detection limit and the maximum of the master curve). The functional sensitivity is 0.02 ng/mL. Values below the detection limit are reported as < 0.01 ng/mL. Values above the measuring range are reported as > 25.0 ng/mL (or up to 250 ng/mL for 10-fold diluted samples).

Lower detection limit

0.02 ng/mL

The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1+2 SD, repeatability study, n=21).

Expected values

	N	5th perc. ng/mL (95% Confidence interval)	Median ng/mL (95% Confidence interval)	95th perc. ng/mL (95% Confidence interval)
Healthy men				
	207	1.37 (0.296 - 1.87)	5.01 (4.33 - 5.69)	12.5 (11.1 - 16.8)
Healthy women (years)				
20 - 24	77	1.54 (0.831 - 1.77)	4.15 (3.65 - 4.47)	9.58 (7.26 - 11.83)
25 -29	121	1.11 (0.821 - 1.72)	3.86 (3.17 - 3.96)	9.24 (7.33 - 10.76)
30 - 34	98	0.615 (0.458 - 0.915)	2.561 (2.110 - 3.281)	7.77 (6.34 - 9.62)
35 - 39	95	0.417 (0.127 - 0.827)	1.986 (1.54 - 3.33)	6.13 (4.97 - 8.26)
40 - 44	93	0.073 (0.022 - 0.255)	0.98 (0.717 - 2.040)	3.08 (2.45 - 6.37)
45 - 50	84	0.041 (0.019 - 0.180)	0.375 (0.28 - 0.45)	1.95 (1.76 - 3.88)
PCOS women				
	177	2.56 (1.48 - 2.95)	7.01 (6.5 - 7.5)	17.41 (13.6 - 21.2)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using DiaSino reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability*		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human Serum 1	0.1	0.005	5.00	0.009	9.0
Human Serum 2	0.44	0.024	5.46	0.03	6.82
Human Serum 3	3.11	0.146	4.71	0.20	6.43
PC Universal 1	1.63	0.088	5.38	0.09	5.52
PC Universal 2	7.86	0.321	4.09	0.42	5.34

*Repeatability = within-run precision

Method comparison

A comparison of the DiaSino AMH assay (y) with the Elecsys AMH (x) using 129 clinical samples gave the following correlations:

Linear regression

y = 1.074 x + 0.044

r = 0.9742

The sample concentrations were between approx. 0 and 22 ng/mL.

Functional sensitivity

0.025 ng/mL

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Hook effect

There is no high-dose hook effect at AMH concentrations up to 500 ng/mL.

References

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Symbols

IVD **In vitro diagnostic medical device**	8°C / 2°C **Temperature limit**	**Consult instructions for use**	REF **Catalog number**
LOT **Batch code**	**Date of manufacture**	**Use-by date**	Σ **Contains sufficient for <n> tests**
Manufacturer	**Do not use if package is damaged and consult instructions for use**	**European Conformity**	EC REP **Authorized representative in the European Community**

diasino

DiaSino Laboratories Co., Ltd
No.68 Jingnansi Road National Eco & Tech Development Area
Zhengzhou 450000, P.R. China. info@diasino.com

EC REP **CMC Medical Devices & Drugs S.L**
C/Horacio Lengo Nº 18, CP 29006 Málaga, Spain. Tel: +34 9512 14054

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